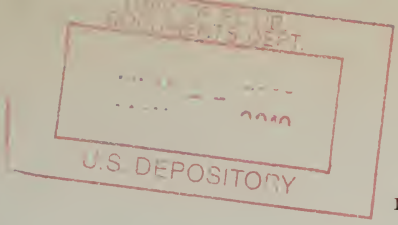

Issued June 22, 1910.

United States Department of Agriculture,

BUREAU OF CHEMISTRY—Circular No. 59.

H. W. WILEY, Chief of Bureau.

THE DESTRUCTION OF THE ENZYM INVERTASE BY ACIDS, ALKALIS, AND HOT WATER.

By C. S. Hudson and H. S. Paine,

Assistant Chemists.

MEASUREMENTS OF THE RATE OF DESTRUCTION.

In a previous publication[a] it was shown that invertase is destroyed by both acids and alkalis. At the temperature of 30° C. the destructive action became noticeable first at the acid concentration of 0.01 normal and rapidly increased with the acid strength, becoming almost instantaneous at 0.05 normal; the alkaline destruction began a little below 0.01 normal and became almost instantaneous at 0.045 normal. It is to be supposed that at lower temperatures these rates will all be smaller and that at such temperatures a stronger acidity or alkalinity will be required for a noticeable destructive action. On the other hand, at higher temperatures the rates of destruction will doubtless be greater and the destructive action will be noticeable for weaker concentrations of acidity and alkalinity. At a sufficiently high temperature the acid and alkaline ions of water itself will doubtless cause a noticeable destructive action of pure water on invertase. It has long been known that hot water destroys invertase and other enzyms; as these views appear to correlate this destruction by hot water with the destruction by acids and alkalis at low temperatures, measurements were made for the purpose of tracing the destructive action of these three agents at different temperatures, in order to learn in what manner the actions are related. The measurements were made by the procedure that was described in the former publication; the results being recorded in Table 1 and shown graphically in figure 1. The recorded rates of destruction are the velocity-coefficients of the unimolecular destruction reaction, multiplied by 1,000, the units of measurement being minutes and decimal logarithms.

[a] U. S. Dept. Agr., Bureau of Chemistry Cir. 55.

TABLE 1.—*Rates of destruction of invertase by acids, alkalis, and water at various temperatures.*

Temperature.	Concentration of hydrochloric acid.	Rate of destruction.	Concentration of sodium hydrate.	Rate of destruction.
°C.				
0	0.03 normal	1	0.03 normal	2
	.04	3	.04	5
	.06	9	.05	17
	.08	34	.06	42
	.10	99	.08	125
15	.03	3	.03	9
	.04	10	.04	38
	.05	19	.05	136
	.06	55		
30	.015	1	.01	3
	.02	5	.02	11
	.03	42	.03	50
	.04	96	.04	245
	.05	365		
45	.01	1	.01	12
	.02	26	.02	41
	.03	772 (?)	.025	128
60	.005	7	.001	14
	.0075	18	.0025	146
	.01	152	Distilled water	1
65	.002	233	.0001	91
	.003	301	.0002	210
			Distilled water	74

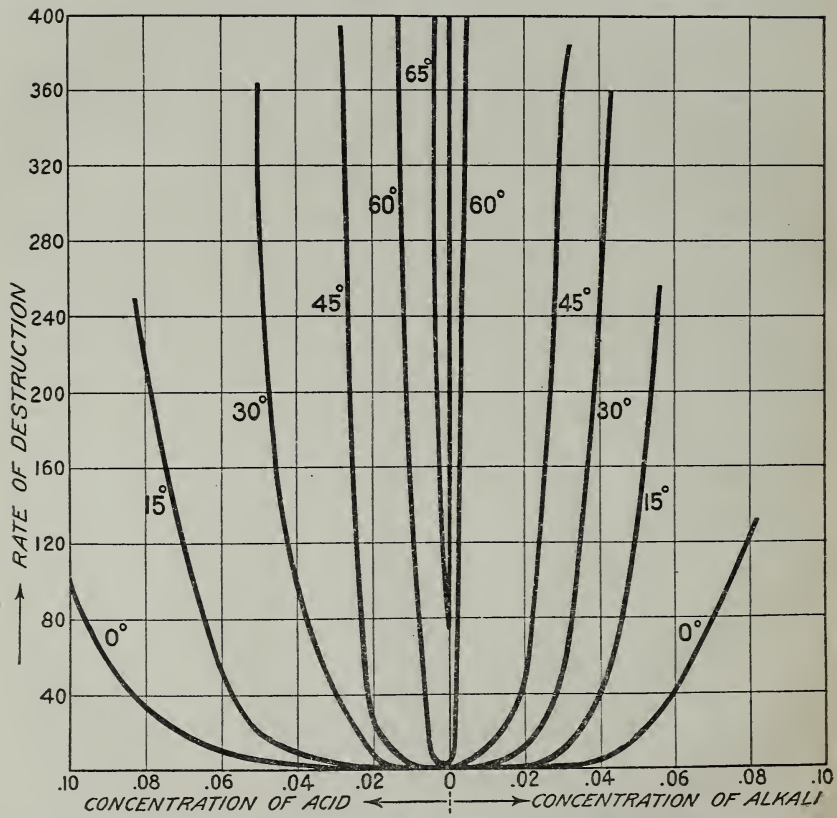

FIG. 1.—The rates of destruction of invertase by acids, alkalis, and water, at various temperatures.

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The results may best be interpreted from a consideration of the figure; it is seen that as the temperature is raised the rate of destruction by acids and alkalis increases until finally at or about the temperature of 60° C. distilled water itself slowly destroys invertase, and at 65° the destruction by water is quite rapid. It is evident that the destruction of invertase by hot water is due to the same cause as is its destruction by acids and alkalis. The latter reactions are doubtless hydrolyses of the complex enzym molecule and it is therefore to be concluded that *the destruction of invertase by hot water is caused by a hydrolysis of the enzym.* This conclusion doubtless applies to other enzyms also. As far as is known this is the first evidence offered to explain the cause of the well known destruction of enzyms by hot water. This point of view explains why dry enzym preparations can be heated without destruction to temperatures over 100° C. in case no water is present; the hydrolysis does not then take place.

THE INFLUENCE OF TEMPERATURE IN INCREASING THE RATES OF DESTRUCTION.

In Table 2 the rates of destruction in the same medium at different temperatures are compared, and in the last column the coefficient which shows how many fold the rate increases for 10° rise in temperature is recorded. Some of the very large and very small rates do not agree with the general average in showing a coefficient of the value of 2 to 4, but the limits of error in these cases are larger. The average value of the coefficient is 3.1, which agrees with the general observation that this factor for most chemical reactions varies between 2 and 4. The hydrolytic destruction of invertase by acids, alkalis, and hot water thus falls in with the common types of chemical reactions.

TABLE 2.—*The temperature coefficient of the destruction.*

Temperature interval.	Concentration.	Rates.	Coefficient for 10° rise.
°C.			
0–30	0.04 HCl	3– 96	3.2
0–15	.06 HCl	9– 55	3.3
0–30	.03 NaOH	2– 50	2.9
0–30	.04 NaOH	5–245	3.7
0–15	.05 NaOH	17–136	4.0
30–45	.02 HCl	5– 26	3.0
30–45	.01 NaOH	3– 12	2.5
30–45	.02 NaOH	11– 41	2.4
Average			3.1

THE PROTECTIVE ACTION OF FRUCTOSE AGAINST THE DESTRUCTION OF INVERTASE.

Recently the authors[a] have shown the very marked effect of cane sugar in protecting invertase from destruction by alcohol; experiments will now be described which show that fructose shares with cane sugar this remarkable property, and also protects invertase from destruction by acids and alkalis. The latter protective action has not yet been tested for cane sugar. The experiments were made by the usual procedure, the rate of destruction being measured first in the absence of fructose and then with it present in the concentrations of 2.7, 5.4, and 10.9 per cent. The data are recorded in Table 3 and the action of fructose in protecting invertase from acid destruction is shown in figure 2.

TABLE 3.—*The action of fructose in protecting invertase from destruction by acids, alkalis, and hot water.*

Temperature.	Concentration of destroyer.	Concentration of fructose.	Rate of destruction.
°C.			
30	0.04 N. HCl	0.0	100
		2.7	26
		5.4	12
		10.9	2
30	0.03 N. NaOH	0.0	100
		2.7	3
		5.4	3
		10.9	4
30	50 per cent alcohol	0.0	100
		2.7	1
		5.4	1
		10.9	1
61	Distilled water	0.0	100
		2.7	32
		5.4	16
		10.9	24

The rates of destruction given in the table are expressed as per cent of the rate for the destroyer when no fructose is present. The rates actually found, expressed as velocity-coefficients of the unimolecular destruction reaction, using minutes and decimal logarithms, are 0.04 normal hydrochloric acid, 0.096; 0.03 normal sodium hydroxid, 0.050; 50 per cent alcohol, 0.85; and water, 0.0052.

It will be seen from the figure that the protective action of fructose in the case of hydrochloric acid is not at all proportional to the concentration of the sugar but approaches a limiting value asymptotically. The limiting value for the protection seems to have been reached in the case of the alkaline solutions and the alcohol with only 2.7 per cent fructose; this is probably also true for the protection from hot

[a] U. S. Dept. Agr., Bureau of Chemistry Cir. 58.

water because the measurements in this case are very difficult to perform accurately, and the values found—32, 16, and 24—do not differ far beyond the possible errors.

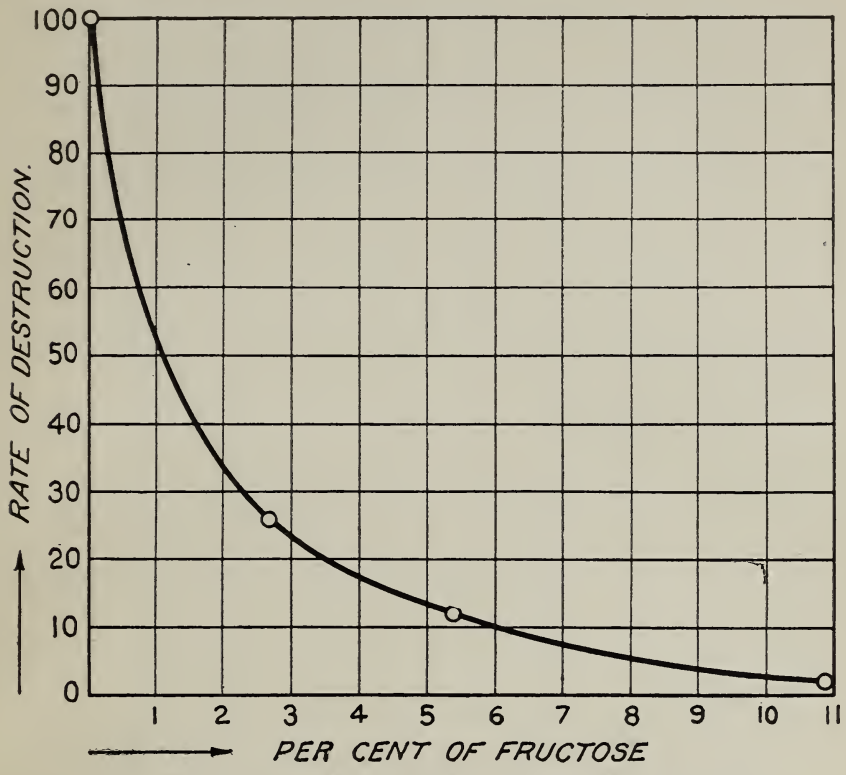

FIG. 2.—The action of fructose in protecting invertase from destruction by acid.

These results on the protection of invertase by fructose indicate that the enzym forms a combination with the sugar which is more resistant to the destructive action of acids, alkalis, hot water, and alcohol than is invertase itself. They also open up a way in which this compound can be investigated.

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